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Evaluation of response to chemotherapy in patients affected with non-small cell lung cancer by means of three tumour markers elaborated by discriminant analysis

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Chemotherapy is the most effective treatment for inoperable patients (70%) affected with non-small cell lung cancer (NSCLC). The early detection of tumour progression is mandative in order to promptly shift these patients towards salvage or supportive therapy.

The present authors investigated the clinical value of a panel of tumour markers, elaborated by means of discriminant analysis, as a follow-up indicator for the detection of tumour progression. The serum levels of tissue polypeptide antigen (TPA), CYFRA-21.1, neuron-specific enolase (NSE) and carcino-embryonic antigen (CEA) were determined before chemotherapy and after three cycles of treatment. Discriminant analysis generated a formula (canonic variable) which correctly classified the 87·8% of the 74 subjects (86·1% of the 36 progressive diseases and 89·5% of 38 non-progressive diseases).

This approach produces an algorithm able to calculate a progression score in NSCLC patients which can be helpful for following-up care and therapy control of these patients.

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Introduction

Adenocarcinoma (LADC), squamous cell carcinoma (SQCLC) and large cell carcinoma (LCLC) have identical biological behaviour and therapeutical approaches, and are collectively termed non-small cell lung cancer (NSCLC). This accounts for 85% of all newly diagnosed lung cancers (1). Although surgery offers the best probability of cure for NSCLC, less than 30% of these patients are candidates for radical resection (2). In subjects with locally advanced and/or disseminated NSCLC and/or with medical contraindications to surgery, chemotherapy represents the only treatment modality.

Unfortunately, the results of combination chemotherapy in NSCLC remain disappointing (the response rates ranging between 30 and 50%) (3). Furthermore, multidrug combinations have shown only a slight advantage when compared with best supportive care, but at the expense of a higher toxicity (4,5). Therefore, patients treated with chemotherapy need to be carefully monitored in order to prevent continuation of ineffective treatments.

In addition to standard restaging procedures (6), the availability of simple laboratory tests that reflect the changes of the total tumour load may be helpful.

Serum tumour markers have been used in lung cancer for monitoring treatment and predicting

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TABLE 1. Clinical characteristics of the patients employed to (a) generate and (b) validate the canonic variable

Histology	Patients	Sex M/F	Age (years) Median	Age (years) Range	Stages I	Stages II	Stages IIIa	Stages IIIb	Stages IV
(a)									
LADC	14	7/7	63	41–80	0	0	3	5	6
SQCLC	26	15/11	62	45–73	4	3	6	4	9
LCLC	3	2/1	65	63–68	0	0	2	0	1
(b)									
LADC	12	6/6	59	46–78	1	1	2	3	5
SQCLC	16	10/6	63	46–74	0	3	3	5	5
LCLC	3	2/1	66	61–67	0	1	1	0	1

LADC, adenocarcinoma; SQCLC, squamous cell lung carcinoma; LCLC, large cell lung carcinoma.

relapse (7). Otherwise, the literature does not report clear indications as to the clinical value of single tumour marker determination during follow-up of patients with NSCLC (8–10). Recently, many studies have shown that the employment of two or more markers, used in combination as a marker panel, can improve their clinical value for monitoring of NSCLC patients (11–13). Furthermore, computerization has made possible the employment of statistical methods to evaluate the role of the different markers in relation to each other and weighted together.

This study represents an attempt to optimize the use of some commonly investigated tumour markers (tissue polypeptide antigen, TPA; CYFRA-21.1; neuron-specific enolase, NSE; and carcino-embryonic antigen, CEA) elaborated by means of discriminant analysis, in order to distinguish progressive disease (PD) from non-progressive disease (NPD) in treated NSCLC patients at the time of restaging.

Patients and Methods

PATIENT POPULATION

From July 1993 to June 1994, a group of 43 consecutive, unselected and previously untreated patients with inoperable NSCLC was evaluated.

A second group of 31 patients affected with inoperable NSCLC was subsequently enrolled to validate the discriminant ability of the canonic variable (CV) generated in the first group.

Pre-treatment staging for all patients was performed according to the guidelines of the American Joint Committee on Cancer (14).

Table 1(a,b) shows the main clinical characteristics of the patients of the two groups. No differences were found between the two groups of patients. Candidacy for surgical resection was excluded on the basis of tumour stage, pulmonary function and comorbid disease. Patients with advanced NSCLC or medical contraindications to surgery were referred to chemotherapy.

All patients received chemotherapy with MVP (mitomycin, 10 mg m^{-2} i.v. on Day 1, vindesine 3 mg m^{-2} on Days 1 and 15, and cisplatin 75 mg m^{-2} on Day 1) every 4 weeks. After the third cycle of chemotherapy, the patients were re-assessed with chest X-ray, radionuclide bone scan and computed tomography of the brain, chest and abdomen.

Tumour volume was defined by multiplying the largest diameter by the greatest perpendicular diameter on chest X-ray.

A complete response (CR) was defined as complete disappearance of all objective clinical evidence of disease; a partial response (PR) was defined as a decrease in tumour volume by

greater than 50%; a minor response (MR) was a decrease by 20–50% of pre-treatment size; no change (NC) was a <20% decrease in tumour size; and progressive diseases (PD) was any increase in tumour volume or the onset of new lesions. Response to chemotherapy was assessed without knowledge of tumour marker levels.

TUMOUR MARKERS

The serum tumour marker levels were determined before chemotherapy and at the time of restaging. The sera of the patients, obtained by venipuncture and stored at −40°C, were tested for CEA (Sorin Biomedica, Saluggia, Italy), TPA (Byk Sangtec, Cormano, Italy), NSE (CIS Diagnostici, Vercelli, Italy) and CYFRA-21.1 (a cytokeratin antigen, CIS Diagnostici, Vercelli, Italy). Samples with evident haemolysis were not included because they were shown to give increased assay values (15). The tests, based on radioimmunoassay techniques, were evaluated in duplicate according to the manufacturers' instructions.

STATISTICAL ANALYSIS

Non-parametric tests were used for the single markers due to their distributions. The Kruskall-Wallis one-way analysis of variance was used to compare the different groups, and the Spearman Rank correlation test was used to calculate the correlation coefficients.

Logarithmic transformation was needed for the value of each tumour marker to normalize its distribution.

Discriminant analysis was performed on a computer using the BMDP program (BMDP statistical software, University of California, U.S.A., P7M module) (16,17).

This multiparametric test permits the generation of CV that can separate two groups. The CV is a score obtained by adding together the levels of the variables selected, multiplied by appropriate coefficients, negative or positive. The CV associates the different discriminant abilities of the various parameters, contributing to the final overall classification. The program selects the most useful parameters for the maximum discrimination, eliminating the highly correlated variables. The CV is standardized (by means of a constant number) to zero when the patient cannot be classified in a group.

Since the formula directly derives from the data of the group selected, overestimation cannot be ruled out (18). To overcome this problem, the authors used; (1) a jack-knifed approach (i.e. each patient was evaluated by a CV obtained after exclusion of the same patient's data); and (2) a validation group (a second group of patients, enrolled subsequently and not employed to generate the first algorithm).

Results

A group of 43 NSCLC patients (generation group) [Table 1(a)] was evaluated before chemotherapy and at the time of restaging. Twenty-three PDs and 20 NPDs (two CRs, two PRs, two MRs and 14 NCs) were found. This group was used to generate a CV on the basis of the tumour marker levels measured before therapy and at the time of restaging to evaluate which tumour markers could be able to distinguish, combined in a single score, the patients with PD from those with NPD.

Table 2(a) reports the differences between the median levels and ranges observed for the individual variables in the generation group subdivided for PD or NPD. For brevity, only the variables selected during the subsequent discriminant analysis are shown. These discriminant factors were represented by the serum CEA and TPA levels, measured at the time of restaging, and the NSE and TPA ratios calculated by dividing the post-treatment by the pre-treatment levels, respectively. It must be emphasized that all marker values were significantly modified when comparing PD and NPD groups.

The formula to calculate the CV score was as follows: $CV = (\text{Ln CEA} \times 0{\cdot}11684) + (\text{Ln TPA} \times 0{\cdot}64461) + (\text{Ln TPA-ratio} \times 0{\cdot}65951) + (\text{Ln NSE-ratio} \times 1{\cdot}01991) - 3{\cdot}50395$, where all the coefficients are positive because their increases augment the progression probability.

Tables 1(b) and 2(b) present the data of another group of 31 NSCLC patients (validation group) collected after the elaboration of the discriminant analysis. In this validation group, all markers were significantly modified between PD and NPD (the only exception was the post-treatment CEA).

TABLE 2. Medians and ranges of the variables selected (a) generation group and (b) validation group

		CEA (ng ml^{-1})	TPA (U ml^{-1})	TPA-R —	NSE-R —
(a)					
PD (23)	Median	6·8	268	1·7	1·1
	Range	0·5–100	47–3000	0·3–5·8	0·5–2·1
NPD (20)	Median	3·45	67·5	1·1	0·9
	Range	0·4–48	10–414	0·1–1·8	0·4–2·3
	P	0·014	0·000048	0·000028	0·011
(b)					
PD (13)	Median	11	199	1·3	1·5
	Range	2·1–70	29–1178	0·5–3·8	0·7–3·9
NPD (18)	Median	3·9	70·5	0·68	1·04
	Range	2·1–22	16–552	0·1–1·7	0·5–3·1
	P	0·09	0·019	0·003	0·03

PD, progressive disease; NPD, non-progressive disease; CEA, carcino-embryonic antigen; TPA, tissue polypeptide antigen; NSE, neuron-specific enolase. Patient numbers are reported in parentheses. Post-treatment/pre-treatment ratio values are indicated by -R after the marker name.

Considering all the patients of the two groups together, only a low correlation coefficient was recorded between post-treatment CEA *vs.* post-treatment TPA (R=0·25; P=0·03). Carcino-embryonic antigen and TPA showed a high correlation with CYFRA-21.1 (data not shown).

Figure 1 shows the individual CV values for each patient, and patients' classifications at the time of restaging. The rate of correct classification was 88·4% for the generation group and 87·1% for the validation group. The overall classification was 87·8%. Figure 1 shows the lack of differences between the generation and validation groups.

No errors were observed for the two CRs and the five PRs. Only one error was found from six MRs (16·6%) and five of the 36 PDs (13·8%).

The CR+PR CV levels, calculated on 74 patients, were statistically different when compared to MR+NC subjects (P=0·03). In addition, the CV levels of the MR+NC sera of the last two groups were significantly different from PD CV levels (P<0·00001). In all patients, the significances calculated for CV were always better than those found for the individual markers included into the CV formula (data not shown). The CV was not correlated to histology, age or sex. On the contrary, CV was related to the pre-treatment stage of the patients, increasing from Stage 1 to Stage IV (P=0·0006).

Discussion

The present study was intended to evaluate whether a statistical procedure could optimize the use of a panel of tumour markers, and provide a practical test for monitoring the response to chemotherapy in NSCLC patients.

For this purpose, four tumour markers, routinely determined for lung cancer follow-up (7–11,13,19–22), were selected and their pre- and post-treatment levels were elaborated by means of discriminant analysis.

This approach is currently applied in taxonomy and epidemiology. The fundamental goal

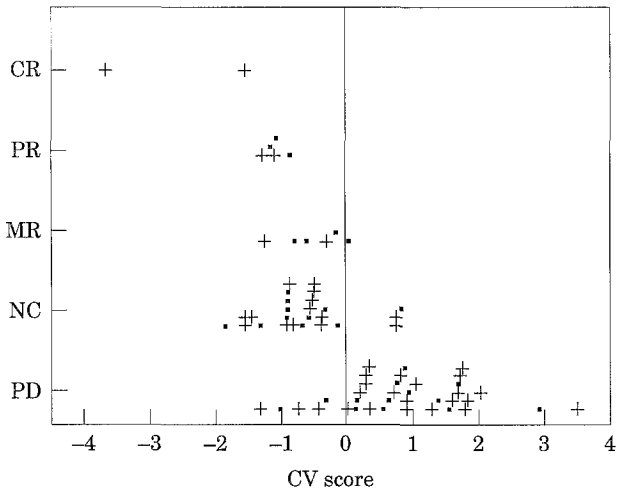

FIG. 1 Discriminant analysis performed on 74 non-small cell lung cancer patients (+, generation group; ▪, validation group) classified as 36 progressive diseases (PD) or 38 non-progressive diseases (NPD) at the restaging time (y axis). The NPD patients included two complete responses (CR), five partial responses (PR), six minor responses (MR) and 25 no changes (NC). A canonic variable (CV, x axis) has been generated, able to correctly classify 65 of 74 subjects (87·8%). Variables included in the CV, after logarithmic transformation, were tissue polypeptide antigen (TPA) and carcino-embryonic antigen values plus the post-treatment/pre-treatment ratios of TPA and neuron-specific enolase.

with which the method is concerned is the allocation of an individual to one of two or more distinct groups. This allocation is obtained on the basis of linear combination of variable measurements made on the individuals, that will discriminate between the *a priori* defined groups, minimizing the misclassification rate. Several studies are reported in the medical literature in different fields employing this method (23–27).

In the present study, the CV was generated including TPA values, TPA ratios, NSE ratios and CEA levels as useful variables for the final classification of the patients as PDs or NPDs.

It has to be emphasized that NSE was also selected as a useful variable. Although this enzyme is generally considered to be the best marker for small cell lung cancer (SCLC), it has also been employed for predicting response to chemotherapy in NSCLC patients (28,29).

Many authors have shown the reliability of CYFRA-21.1 as a marker for NSCLC (10,20,22,24), but the present analysis excluded this cytokeratin from the list of selected variables. This occurrence is probably related to the lower significant differences of CYFRA-21.1 values found between the two groups of subjects, and to the strong correlation with TPA and CEA.

Of the 43 subjects in the generation group, 88·4% were correctly distinguished by the jackknifed classification approach (16). A further validation was obtained on a second group of 31 patients, confirming the reliability of this formula for classifying patients not previously used for the CV generation. The analysis of the CV distribution among the five different types of response (CR, PR, MR, NC and PD), as evaluated by means of restaging techniques, showed that PDs are clearly distinguishable from NC, indicating that PD is characterized by an evident CV increase.

Imaging data represent the standard procedure for the objective evaluation of therapeutic activity of anti-neoplastic drugs. Some of them, such as computerized tomographic scan and radionuclide bone scan, are extremely reliable procedures, but are resource demanding and time-consuming for the patient, and therefore not used routinely in many peripheral clinical centres. On the other hand, chest X-ray is a simple, routine and inexpensive test. Nevertheless, the precision of this technique is often hampered by lack of measurable lesions when the tumour image is obscured by atelectasis, pneumonia, pleural effusion and normal intrathoracic structures (30). For such cases, relatively simple serological tests may be helpful to assess the response to chemotherapy. Radioimmunological or immunoenzymatic determination of tumour markers is now available everywhere, and the automatization of these tests easily permits their measure in a large number of specimens. The increasing quality of the reagent standardization produces reliable results due to data reproducibility both intra- and interassay.

On this basis, and until more powerful tumour markers are developed, this CV seems to be useful as a monitoring test to detect disease progression and promptly prevent continuation of ineffective toxic treatment, especially in patients with poor cardiorespiratory functions, often worsened by chemotherapy.

Due to the small number of patients enrolled in this study, a larger group of NSCLC subjects will be analysed in the future. Therefore, another study has been planned to confirm the promising results of the present work. Also, the authors will evaluate whether the use of a tumour marker panel, determined after the first cycle of chemotherapy, may allow an earlier detection of relapse, preceding the clinical evidence of tumour recurrence. In addition, this future study will explore a possible relationship between CV levels and patient survival.

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